

# Potential Heterotic Groups in Hop as Determined by AFLP Analysis

M. Shaun Townsend\* and John A. Henning

## ABSTRACT

Hop (*Humulus lupulus* L.) is a perennial, dioecious species in which the female inflorescence (cones) are harvested and used in the beer-brewing process to impart bittering and/or flavoring to beer. Hop breeders have typically utilized clonal selection and hybridization to develop new cultivars. The use of genetically diverse parents for the purpose of capturing heterosis in the offspring has received little attention from hop breeders. The objective of this research was to assign male ( $N = 80$ ) and female ( $N = 26$ ) hop genotypes into potential heterotic groups using AFLP-generated molecular markers. The six AFLP primers used in this study amplified 550 total fragments, of which 490 (89.1%) were polymorphic. A genetic distance (GD) matrix was computed from the binary data matrix, and groupings and summary statistics were calculated from the GD matrix. Two major clusters were observed, one composed primarily of European hops, while the second group was composed primarily of European-wild American hybrids. The two major clusters were further subdivided into 13 smaller clusters (two female, nine male, two male and female) based on a qualitative analysis. These results suggest potential parental combinations for hop researchers and breeders to study heterosis in hop.

HOP IS A PERENNIAL, climbing, dioecious species in which the female inflorescence (cones) are harvested and used in the beer-brewing process to impart bittering and flavoring to the final product. Hop cultivars are developed for two primary uses; the bittering cultivars are used as bittering agents in beer brewing, while the aroma cultivars are used to impart flavor and aroma in beer.

Early hop improvement efforts were mainly conducted by growers selecting the most desirable genotypes for use in a given geographic area. Since hop is a vegetatively propagated species, traits can be easily fixed and cultivars are typically released after a single selection cycle (Haunold, 1981; Neve, 1991).

Traits of interest in hop production include high  $\alpha$ -acid content for bittering hops, low or moderate  $\alpha$ -acid, and a desirable combination of essential oils for aroma hops, storage stability (i.e., low hop storage index), low cohumulone (an  $\alpha$ -acid homolog), high yield, and resistance to downy [*Pseudoperonospora humuli* (Miyabe et Takah.) G. Wilson] and powdery (*Podosphaera macularis* Braun & Takamatus) mildews. Genetic variation was found for  $\alpha$ -acid,  $\beta$ -acid, flower weight, and number of lupulin glands in 20 male hop clones from 17 different pedigrees (Brooks and Likens, 1962). Alpha-acid and

$\beta$ -acid was negatively correlated among 112 female ( $r = -0.78$ ), and among 74 male hop genotypes ( $r = -0.85$ ). Additive genetic variation was detected for yield,  $\alpha$ - and  $\beta$ -acid content, cohumulone, and hop storage index for both males and females in a North Carolina Design I analysis of 25 yr of USDA historical data (Henning et al., 1997a). They also reported negative genetic correlations between  $\alpha$ -acid and  $\beta$ -acid content, and between  $\beta$ -acid and hop storage index, and positive genetic correlations between cohumulone and yield, and cohumulone and hop storage index. Genetic variation in hop has also been reported for essential oil concentration (Henning et al., 1997b) and mineral concentration (Keller and Likens, 1955).

Several molecular marker tools have been employed to assess genetic diversity in hop, including amplified fragment length polymorphism (AFLP) (Hartl and Seefelder, 1998; Seefelder et al., 2000; Townsend et al., 2000), random amplified polymorphic DNA (RAPD) (Pillay and Kenny, 1996; Seefelder et al., 2000), and microsatellites (Jakse et al., 2001). Previously published hop genetic diversity reports based on molecular marker data have focused on female genotypes. In an analysis of 41 female hop accessions by AFLP and microsatellites, two distinct germplasm groups were observed representing American and European ancestry (Jakse et al., 2001). In an AFLP analysis of 86 female and four male hop accessions, two major groups were identified that corresponded to European and European  $\times$  wild American hybrids, and these were subdivided into numerous smaller subgroups (Seefelder et al., 2000).

Predicting hybrid performance, or heterosis with molecular markers has not been pursued in hop but has received research attention in other crop species. Genetic distance measured by pedigree, plant morphology, or molecular markers does not necessarily correlate in a linear fashion with heterosis although Lanza et al. (1997) did find a positive correlation between RAPD-based GD and maize single-cross hybrid grain yield. However, GD has proven useful in grouping related genotypes together that exhibit heterosis when crossed to genotypes in unrelated groups (Cheres et al., 2000; Fabrizio et al., 1998; Sant et al., 1999).

Because accurate pedigree information is not available for some hop genotypes, molecular markers could be used to assign genotypes to heterotic groups. Once assigned to groups, genotypes could be crossed in a systematic manner to establish heterotic combinations. The objective of this research was to place 106 male and female hop genotypes into potential heterotic groups via AFLP-based GD estimates.

M.S. Townsend, Dep. of Crop and Soil Science, Oregon State Univ., and J.A. Henning, National Forage Seed Production Research Center, USDA-ARS, Corvallis, OR 97331. Received 19 Dec. 2003. \*Corresponding author (townsenm@onid.orst.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

**Abbreviations:** GD, genetic distance.

## MATERIALS AND METHODS

### Plant Materials

One hundred six hop accessions representing a broad range of genetic backgrounds were chosen for evaluation (Table 1). These accessions are part of the USDA-ARS hop breeding and genetics program's collection and include both male ( $N = 80$ ) and female ( $N = 26$ ) genotypes from wild American and European ancestry. Male genotypes were grown in a field nursery near Corvallis, OR, and were managed similar to commercial hop yards in the Pacific Northwest USA with the exception that pesticides were not applied. Female genotypes were grown in a greenhouse facility in Corvallis, OR, using a commercial soil mix without supplemental lighting, and were watered, fertilized, and pests controlled as needed.

### AFLP Analysis

Leaf tissue was harvested, rinsed with deionized water, blotted dry on a paper towel, and stored at  $-80^{\circ}\text{C}$ . Frozen samples were freeze-dried at  $-40^{\circ}\text{C}$  for 24 to 26 h, switched to  $-20^{\circ}\text{C}$  for an additional 24 to 26 h, and then stored at  $-20^{\circ}\text{C}$  before analysis. Approximately 100 to 600 g of freeze-dried tissue was used in DNA extraction following the protocol of Kidwell and Osborn (1992). The AFLP protocol used was previously described by Townsend et al. (2000) and primer sequences are listed in Table 2. Primer combinations used in selective amplification were eAAC-mCAC, eAAC-mCAG, eAAC-mCTC, eAGC-mCAG, eAGC-mCTC, and eACC-mCAC. Gel bands were detected on an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA).

### Data Analysis

Genographer software (Benham et al., 1999) was used to score each gel. A binary data matrix was created from the scored images and genetic similarity estimates were computed using the equation provided by Dice (1945). The resulting genetic similarity matrix was converted to a GD matrix ( $1 - \text{similarity value}$ ) and a dendrogram computed using Ward's (1963) method in the Cluster Analysis module of Statistica software (Version 5.0, StatSoft, Inc., Tulsa, OK). Cluster determination was based on sex, and by studying known pedigree information and previous hop taxonomic research (Small, 1978, 1980). The average GD estimate between two clusters was computed by averaging GD estimates from all possible combinations of pairs between the two clusters. The overall GD estimate for each cluster was computed by averaging all of the between-cluster GD estimates.

## RESULTS

### AFLP

The six AFLP primers used in this study amplified 550 total fragments, of which 490 (89.1%) were polymorphic (Table 3). The eAAC-mCTC primer amplified the most fragments (110) while eAAC-mCAC amplified the least (72). Primer eAAC-mCAC also generated the least amount of polymorphism (77.8%) while eAGC-mCAG exhibited the most polymorphism (98.9%).

### Clustering

A dendrogram was computed based on GDs calculated from the AFLP analysis (Fig. 1). Potential heterotic groups were delineated based on sex, and by studying

known pedigree information and previous hop taxonomic research (Small, 1978, 1980). Using these criteria, two major groups (A, B) were observed, and these were subdivided into 13 smaller clusters (A1–A4, B1–B9) (Fig. 1, Table 1).

The two major groups generally corresponded to the two domesticated hop germplasm pools used to develop hop cultivars. Group A contained 21 males and 18 females which were primarily of European descent. In addition, 15 females in group A are considered aroma hops while three females ('Newport', 'Nugget', and 'Yeoman') are considered bittering hops. Most group A males typically had German or Yugoslavian ancestry, had 'Northern Brewer' as the female parent, or were descended from females derived from 'Late Grape'  $\times$  'Fuggle' crosses. Group B was dominated by genotypes with wild American–European hybrid ancestry. Approximately 75% ( $N = 59$ ) of the males analyzed in this study were in group B and many have complex pedigrees. All of the males studied that are known to have 'Comet', 'Cascade', 'Bullion', or 'Late Cluster' as the female parent, or as part of the female parent's ancestry, clustered in group B. Eight females clustered in group B and seven have wild American germplasm in their ancestry. Cascade was the only female in group B not known to have wild American germplasm in its pedigree. However, Cascade was selected from an open-pollinated female, and thus, could have wild American ancestry via the unknown male donor (Brooks et al., 1972).

Group A was subdivided into four smaller clusters, while group B was subdivided into nine smaller clusters (Table 1, Fig. 1). Clusters A1 and B1 contained only females while clusters A2, A3, and B3 to B9 contained only males. Clusters A4 and B2 contained a mix of males and females. The females in cluster A1 were mainly aroma hops derived from European germplasm while the females in cluster B1 are bittering hops that trace to wild American germplasm. The females Comet, Cascade, and Target clustered with six males in B6. Five of the six males in B6 descended from Cascade. Cluster A4 was dominated by Fuggle-derived males and females (Haunold, personal communication; Henning and Haunold, 2003), while A2 contained males developed in Yugoslavia and males descending from Northern Brewer. Cluster A3 contained the accessions from the hop program at Hüll, Germany.

### Genetic Distance

Genetic distance estimates between the 13 proposed subgroups were generally greatest between groups of different ancestry (European vs. wild American–European hybrids). The highest observed estimates were between A1 vs. B6, A3 vs. B6, and A4 vs. B6 (Table 4). Group B6 is comprised of males with a wild American–European hybrid origin while A1, A3, and A4 are predominantly from European ancestry. Not surprisingly, the smallest observed GD estimates were between subgroups within the same major cluster. The smallest GD estimates were observed for A1 vs. A2, B3 vs. B4, and B2 vs. B4 (Ta-

Table 1. List of hop genotypes arranged into proposed heterotic groups based on cluster analysis of AFLP marker data.

Accession	Pedigree
<b>A Group (primarily European ancestry)</b>	
<b>A1 Subgroup</b>	
Wye Saxon (F21282)	Svalof/Bramling Cross/Wye 1-63-42
Wye Viking (F21283)	Svalof/Bramling Cross/Wye 1-63-42
Orion (F21675)	Perle/German 70-10-15
Northern Brewer (F21093)	Brewer's Gold/OY1/Canterbury Golding
Omega (F21667)	Wye Challenger/English male
Challenger (F21043)	Zattler/open pollinated/Northern Brewer/Wye 22-56
Perle (F21227)	Northern Brewer/German 63-5-27
Yeoman (F21498)	Wye 43/69/17 × Wye 25/68/173
Nugget (F21193)	Brewer's Gold/Early Green/Unknown-s/3/Brewer's Gold/East Kent Golding/Bavarian-s†
East Kent Golding (F21680)	Old English cultivar
Spalter Select (F21674)	German 76-18-80/German 71-16-07
Tardif de Bourgogne (F21169)	France landrace
Fuggle N (F21016)	Clonal selection from Fuggle
Saazer 36 (F21521)	Clonal selection from Saazer
Hallertauer Mittlefruh (F21014)	German landrace
<b>A2 Subgroup</b>	
M21089	Yugoslavia selection 5-10
M21400	Native Yugoslavian Male 20P09
M21398	Native Yugoslavian Male 01P04
M21268	Northern Brewer/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
M21090	Yugoslavia Selection 12-17
M21087	Yugoslavia Selection 3-3
M21335	Northern Brewer/Bullion/Zattler-s
M21336	Northern Brewer/Bullion/Zattler-s
M21132	Yakima Cluster/Zattler-s
M21009	Sunshine-s/3/Utah-523-4/Early Green/Unknown-s
<b>A3 Subgroup</b>	
M64035	Zattler-s
M64101	Unknown
M64034	Zattler-s
M64037	Zattler-s
M64036	Zattler-s
M64033	Zattler-s
<b>A4 Subgroup</b>	
Newport (F21736)	(Galena/German 75-5-3/5/(Brewer's Gold/Belgium 31-s/Belgium 31/3/Late Grape/Fuggle/4/open pollinated)
Styrian (F21049)	Yugoslavian selection from Fuggle
U.S. Tettnanger (F21015)	Fuggle
M63011	Late Grape/Fuggle-s/Early Green/Unknown-s
M21692	Late Grape-s/Fuggle/Fuggle-s/3/Late Cluster-s/Fuggle-s/4/Late Cluster-s/Fuggle-s
M21690	Late Grape-s/Fuggle/Fuggle-s/3/Late Cluster-s/Fuggle-s/4/(Late Grape-s/Fuggle/Fuggle-s/3/Late Cluster-s/Fuggle-s
M19047	Elsasser/Fuggle-s
M19007	Brewer's Favorite-s
<b>B Group (primarily wild American-European hybrids)</b>	
<b>B1 Subgroup</b>	
Kitamidori (F21677)	Japan C79-27-01/Japan C79-64-110
Magnum (F21670)	Galena/German 75/5/3
Galena VF (F21699)	Meristem-tip culture from Galena
Galena (F21182)	Brewer's Gold/open pollinated
Brewer's Gold (F21116)	BB1/open pollinated
<b>B2 Subgroup</b>	
M21488	Cascade/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
Comet (F62013)	Sunshine-s/Utah 524-2
M21465	Comet/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
Wye Target (F21112)	Northern Brewer/Wye 22-56/Eastwell Golding/OB79
Cascade (F21092)	Fuggle/Serebrianca/Fuggle-s/3/open pollinated
M21463	Cascade/Yugoslavian 3-3
M21448	Cascade/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
M21428	Cascade/Fuggle-s/Fuggle-s
M21426	Cascade/Fuggle/Fuggle-s
<b>B3 Subgroup</b>	
M21416	Bullion/Zattler-s
M21466	Comet/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
M21360	Cascade/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
M21300	Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
M21303	Bullion/Zattler-s
M21135	Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
M21110	Bullion/Zattler-s
<b>B4 Subgroup</b>	
M21420	Comet/3/Brewer's Gold/Fuggle/Colorado-2-1/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s/4/Northern Brewer/Bullion/Zattler-s
M21417	Comet/3/Brewer's Gold/Fuggle/Colorado-2-1/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s/5/Comet/Bullion/Zattler-s
M21444	Comet/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
M21273	Comet/3/Brewer's Gold/Fuggle/Colorado-2-1
M21415	Brewer's Gold/Early Green/Unknown-s/3/Late Cluster-s/Fuggle-s
M21272	Northern Brewer/Bullion/Zattler-s
M21329	Comet/Bullion/Zattler-s
M21345	Comet/3/Brewer's Gold/Fuggle/Colorado-2-1/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
M21306	Comet/3/Brewer's Gold/Fuggle/Colorado-2-1/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s/5/Comet/Bullion/Zattler-s
M21313	Comet/4/Brewer's Gold/Early Green/Unknown-s/3/Zattler-s
M21109	Brewer's Gold/Early Green/Unknown-s/3/Zattler-s

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Table 1. Continued.

Accession	Pedigree
<b>B5 Subgroup</b>	
M21435	Cascade/Colorado-1-1
M21437	Fuggle/open pollinated
M21487	Cascade//East Kent Golding/Bavarian-s
M21432	Cascade/4/Late Grape-s//Fuggle/Fuggle-s/3/Early Green/Unknown-s
M64105	Fuggle//Wild American/open pollinated
M64102	Wild American/open pollinated
M63015	Brewer's Gold//East Kent Golding/Bavarian-s
M21351	Comet/3/Brewer's Gold//Fuggle/Colorado-2-1/4/Bullion/Zattler-s
<b>B6 Subgroup</b>	
M21461	Comet/3/Brewer's Gold//Fuggle/Colorado-2-1/4/(Brewer's Gold//Early Green/Unknown-s)/3/Zattler-s/5/Comet//Bullion/Zattler-s
M21129	Late Grape-s//Fuggle/Fuggle-s/3/Brewer's Gold/Utah-526-4
M21072	Brewer's Gold/Arizona-1-2
<b>B7 Subgroup</b>	
M21427	Cascade//Red Vine/Fuggle-s
M21425	Cascade//Semsch-s/8-2B yrd
M21184	Unknown
<b>B8 Subgroup</b>	
M52042	Late Grape/Fuggle-s//Late Grape/Fuggle-s
M21462	Cascade//Fuggle/Fuggle-s
M21358	Comet/3/Brewer's Gold//Fuggle/Colorado-2-1/4/Brewer's Gold//East Kent Golding/Bavarian-s
M21339	Comet/3/Brewer's Gold//Fuggle/Colorado-2-1/4/Brewer's Gold//Early Green/Unknown-s/3/Zattler-s
M21076	Comet/3/Brewer's Gold//Fuggle/Colorado-2-1
M51114	Landhopfen-s//Golden Cluster/Fuggle-s/3/Semsch-s/8-2B Yrd
M21603	Cascade//Semsch-s/8-3B yrd
M52047	Striesselspalt//Early Green/Unknown-s/3/Striesselspalt/Late Cluster-s
M58111	Brewer's Gold//Belgian-31-s/Belgian-31/3/Late Grape/Fuggle-s
M21446	Northern Brewer/3/Brewer's Gold//East Kent Golding/Bavarian-s
M21058	Fuggle//Striesselspalt/Late Cluster-s
<b>B9 Subgroup</b>	
M19172	Cat's Tail//Fuggle/Fuggle-s
M21424	Cascade/Late Cluster-s
M19009	Fuggle/Fuggle-s
M19060	East Kent Golding/Bavarian-s
M19046	Late Cluster-s/Fuggle-s
M19061	Late Grape/Fuggle-s
M19037	Fuggle-s/Fuggle-s
M19041	Early Green/Unknown-s
M19036	Late Cluster/Fuggle-s
M19005	Late Cluster-s

† -s denotes seedling from genotype.

ble 4). Subgroups A1 and A2 contain genotypes of European ancestry, while subgroups B2, B3, and B4 contain genotypes with wild American–European hybrid ancestry. Overall, clusters A3, B6, and B9 had the highest average GD estimates with other clusters while clusters B2, B3, and B4 had the lowest (Table 5).

## DISCUSSION

The ultimate goal of this research is to identify possible heterotic groups that hop breeders might exploit for hop improvement. This experiment examined substantially more male hop genotypes than previous molecular diversity studies. The degree of polymorphism detected here is considerably higher than in previous AFLP reports in hop (Hartl and Seefelder, 1998; Seefelder et al., 2000; Jakse et al., 2001), and may be due to different

primer combinations employed or the larger number of male genotypes studied. Many of the female genotypes were developed via clonal selection and are likely more closely related than the male genotypes, which were developed by hybridization.

We found two major groups, European and wild American–European hybrids, which is similar to results reported by Seefelder et al. (2000) and Jakse et al. (2001). Two genotypes did not cluster similarly to earlier reports. Nugget grouped with the European types in our work but with wild American–European hybrids in Jakse et al. (2001) (Fig. 1). Magnum clustered with the European germplasm-based cultivars in work by Seefelder et al. (2000), while Magnum clustered with the European–wild American hybrids in our work, and that of Jakse et al. (2001). Fuggle did not cluster where we expected based on pedigree information and long-term chemical analysis. Thus, the plant we sampled may be mislabeled.

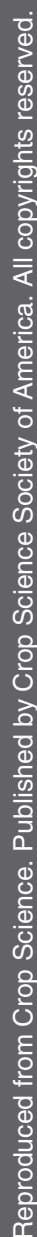
The thirteen subdivisions of the major groups based on AFLP-based GD estimates generally agreed with pedigree data. Female genotype clustering patterns were more obvious than male clustering patterns, although Yugoslavian-bred males clustered together, and males developed in Germany clustered together. Male hop development has received less attention than female de-

Table 2. Oligonucleotide sequences used for AFLP analysis in hop.

<b>Adapter</b>	<i>EcoRI</i>	5'-CTCGTAGACTGCGTACC
	<i>MseI</i>	5'-GACGATGAGTCCTGAG
<b>Preselective amplification</b>	<i>EcoRI</i>	5'-GACTGCGTACCAATTC + A
	<i>MseI</i>	5'-GATGAGTCCTGAGTAA + C
<b>Selective amplification</b>	<i>EcoRI</i> (eAGC)	5'-GACTGCGTACCAATTC + AGC
	<i>EcoRI</i> (eACC)	5'-GACTGCGTACCAATTC + ACC
	<i>EcoRI</i> (eAAC)	5'-GACTGCGTACCAATTC + AAC
	<i>MseI</i> (mCAG)	5'-GATGAGTCCTGAGTAA + CAG
	<i>MseI</i> (mCAC)	5'-GATGAGTCCTGAGTAA + CAC
	<i>MseI</i> (mCTC)	5'-GATGAGTCCTGAGTAA + CTC

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Table 4. Average genetic distance and standard deviation between the 13 proposed hop subgroups estimated from AFLP molecular marker data.

	Groups											
	A1	A2	A3	A4	B1	B2	B3	B4	B5	B6	B7	B8
A2	0.140 ± 0.033											
A3	0.163 ± 0.026	0.168 ± 0.028										
A4	0.163 ± 0.023	0.178 ± 0.037	0.159 ± 0.032									
B1	0.166 ± 0.025	0.196 ± 0.037	0.211 ± 0.025	0.192 ± 0.020								
B2	0.170 ± 0.028	0.173 ± 0.037	0.196 ± 0.024	0.185 ± 0.019	0.161 ± 0.029							
B3	0.166 ± 0.018	0.164 ± 0.019	0.192 ± 0.025	0.187 ± 0.021	0.162 ± 0.023	0.149 ± 0.018						
B4	0.163 ± 0.021	0.153 ± 0.023	0.187 ± 0.027	0.196 ± 0.029	0.162 ± 0.023	0.142 ± 0.020	0.138 ± 0.021					
B5	0.218 ± 0.024	0.212 ± 0.027	0.196 ± 0.035	0.196 ± 0.030	0.188 ± 0.025	0.175 ± 0.025	0.186 ± 0.031	0.187 ± 0.030				
B6	0.254 ± 0.033	0.250 ± 0.035	0.268 ± 0.036	0.255 ± 0.030	0.201 ± 0.034	0.193 ± 0.028	0.202 ± 0.024	0.193 ± 0.031	0.202 ± 0.025			
B7	0.178 ± 0.014	0.175 ± 0.014	0.217 ± 0.025	0.193 ± 0.023	0.170 ± 0.016	0.160 ± 0.023	0.173 ± 0.020	0.172 ± 0.019	0.196 ± 0.026	0.202 ± 0.037		
B8	0.165 ± 0.029	0.163 ± 0.030	0.191 ± 0.032	0.187 ± 0.029	0.183 ± 0.024	0.159 ± 0.024	0.178 ± 0.021	0.167 ± 0.027	0.194 ± 0.028	0.199 ± 0.047	0.172 ± 0.021	
B9	0.213 ± 0.021	0.212 ± 0.027	0.246 ± 0.024	0.227 ± 0.022	0.197 ± 0.023	0.172 ± 0.022	0.203 ± 0.021	0.196 ± 0.021	0.211 ± 0.027	0.213 ± 0.026	0.192 ± 0.018	0.192 ± 0.024

Table 5. Average genetic distance among 13 proposed hop diversity groups as determined by AFLP analysis.

Group	Overall mean	Standard deviation
A1	0.180	0.031
A2	0.182	0.029
A3	0.200	0.031
A4	0.193	0.025
B1	0.182	0.017
B2	0.170	0.017
B3	0.175	0.019
B4	0.171	0.019
B5	0.197	0.012
B6	0.219	0.027
B7	0.183	0.016
B8	0.179	0.013
B9	0.206	0.018

to confirm heterotic groupings in hops and the impact these groupings have on heterosis for important traits.

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